Determination of the cell-free layer in circular PDMS microchannels

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In microcirculation the cell-free layer is believed to reduce the friction between red blood cells (RBCs) and endothelial cells and consequently reduce blood flow resistance. However, the complex formation of the cell-free layer has not yet been convincingly described mainly due to multi-physical and hemorheological factors that affect this phenomenon. In this experimental work, we study the effect of hematocrit (Hct) on the thickness of the cell-free layer in straight circular polydimethylsiloxane (PDMS) microchannels. The channels studied are 73 ± 2 μm in diameter, flexible and circular to mimic blood vessels. The images are captured using confocal microscopy and are post-processed using Image J and MATLAB. The formation of a cell-free layer is clearly visible in the images captured and by using a combination of image analysis techniques we are able to detect an increase in the cell-free layer thickness as Hct decreases.

1 INTRODUCTION

Microfluidic devices are affordable systems that offer the ability to work with minimal sample volumes, short assay times, possibility of parallel operations (lab-on-chip) and efficient control and replication of microenvironments (Beebe et al. 2002). For these reasons, microfluidics has shown great potential to address problems in fields such as engineering and biomedicine, making use of the unique physics at the microscale. Recently, Faivre et al. (2006) made use of the Fahraeus effect to design a microfluidic device for blood separation. The authors were able to create an artificial cell-free layer in their in vitro model by incorporating a geometrical constriction within the microchannel. As such, they have only investigated the effect of model constrictions on the cell-free layer. Very recently, our research group has also performed confocal micro-PIV measurements on the blood flow through straight PDMS microchannels (Lima et al 2008). In that study we used planar PDMS microchannels with a cross section aspect ratio, Height/Width (H/W), of 0.15 and for those particular microchannels no cell-free layer was observed during our experiments. We then increased the cross section ratio H/W up to 0.62 and we observed the formation of a marginal cell-free layer. Our qualitative *in vitro* experiments suggest that the formation of the cell-free layer is enhanced as the cross section ratio (H/W) increases. However, we recognize that the cross section ratio is not the only parameter that contributes for the creation of the cell-free layer. Several other physical and hemorheological factors (such as flow rate, hematocrit, viscosity and cell deformability) need to be addressed in order to make use of the physics of microfluidics to either develop new lab-on-chip devices or optimize the design of existing microfluidic chips.

The main purpose of this paper is to measure the thickness of the cell-free layer *in vitro* using straight circular PDMS microchannels. This experimental study was performed using a confocal microscopy system together with image analysis techniques. This way we expect to gain understanding about the effect of the Hct and channel cross section on the cell-free layer formation.

2 MATERIALS AND METHODS

2.1 Working fluids and microchannel geometry

Four working fluids were used in this study: dextran 40 (Dx40) containing about 3%(3Hct), 13% (13Hct), 23% (23Hct) and 37% (37Hct) by volume of human RBCs. Blood was collected from a healthy adult volunteer and ethylenediaminetetraacetic acid (EDTA) was added in order to prevent coagulation. The RBCs were separated from bulk blood by centrifugation (1500 RPM for 5 minutes) and aspiration of the cell-free and buffy coat. The RBCs were then washed twice with a physiological saline (PS) solution, labeled with a fluorescent dye (CM-Dil, C-7000, Molecular Probes) and subsequently diluted with Dx40 to make up the required RBC concentration. All blood samples were stored hermetical at 4ºC until the experiments were performed at controlled temperature of about 37ºC. All procedures in this work were carried out in compliance with the Ethics Commit-

tee on Clinical Investigation of Tohoku University (Lima 2007).

By using a wire casting technique (Lima 2007; Lima et al. 2009) it was possible to fabricate straight circular PDMS microchannels with a diameter of 73 ± 2 μm (c.f. Figure 1).

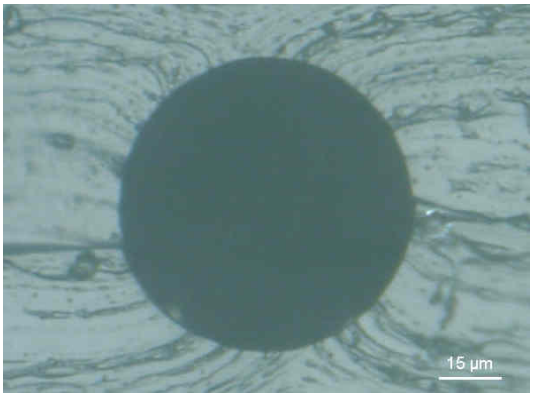

Figure 1. Cross-section of the PDMS microchannel.

2.2 Confocal microscopy set-up

The confocal microscopy system (Lima et al. 2006) used in our experiment consists of an inverted microscope (IX71, Olympus, Japan) combined with a confocal scanning unit (CSU22, Yokogawa, Japan) and a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd, UK) with an excitation wavelength of 532 nm. Moreover, a high-speed camera (Phantom v7.1, Vision Research, USA) was connected into the outlet port of the CSU22 (see Figure 2). The PDMS microchannel was placed on the stage of the inverted microscope and the flow rate of the working fluids was kept constant by means of a syringe pump (KD Scientific Inc., USA). A thermo plate controller was set to 37ºC. All the confocal images were captured in the middle of the microchannels with a resolution of 640×480 pixels, at a rate of 100 frames/s and an exposure time of 9.4 ms. The recorded images were transferred to the computer and then evaluated in Image J (NIH) (Abramoff et al. 2004) using a manual tracking MTrackJ (Meijering et al. 2006) plugin.

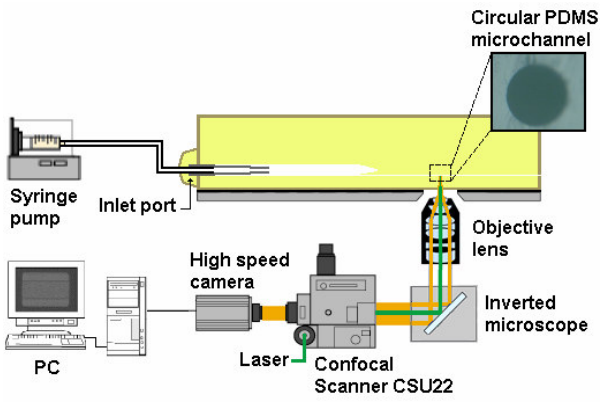

Figure 2. Experimental set-up.

2.3 Image analysis

In this section we present a short description of the two image analysis methods used to determine the cell-free layer thickness.

Method 1

For Method 1, we make use of a number of videos with labelled RBCs flowing on the boundary region of the cell free layer. Using a manual tracking plugin (MtrackJ) available for Image J we are able to track the labelled RBCs located close to the boundary region of the cell-free layer. Figure 3 shows an example of the trajectories of two labelled RBCs flowing close to the boundary of the cell-free layer. The radial position of the tracked RBCs is then determined and the corresponding thickness of the cell-free layer is calculated. Finally, the data is time-averaged.

When the number of labelled RBCs flowing close to the boundary region is not significant, additional analysis is performed by manually measuring the distance from the boundary region to the wall for several points along the microchannel.

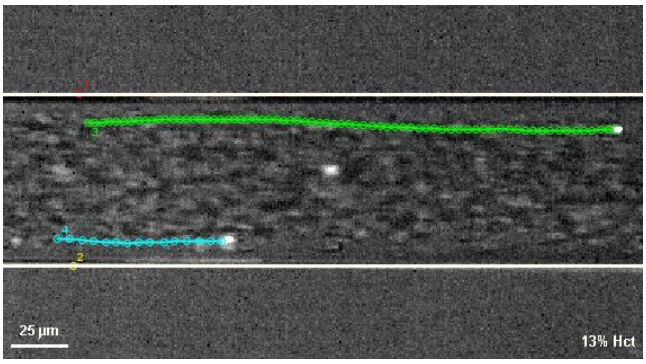

Figure 3. Labeled RBCs flowing close to the boundary region of the cell-free layer.

Method 2

This method uses Image J to detect the thickness of the cell-free layer based on grayscale intensity profiles along the radial direction. The first step consists of converting each image captured to an 8-bit image to be analysed. A new image is then generated using an edge detection plugin available for ImageJ: FeatureJ©. This plugin is made available by Erik Meijering and is based on Canny's algorithm [Meijering 2008] for edge detection. The radial profile of grayscale intensities is determined for each axial position along the channel and the intensity profiles are averaged over the length of the channel visible in the images. Furthermore, a minimum of 50 images is used to compute the average data presented in Section 3. Manual analysis of the averaged intensity profiles allows for the determination of the radial position of the boundaries of the cell-free layer as well as that of the walls.

3 RESULTS AND DISCUSSION

In this section we present some preliminary results concerning the effect of Hct on the cell-free layer obtained by blood flow visualization. We should point out that these are only preliminary results and that additional image analysis to obtain more detailed quantitative measurements of the cell-free layer is currently under way.

Figure 4 shows microscope images taken at the centre plane of a 73 ± 2 µm circular PDMS microchannel. These images show both non-labeled RBCs (halogen illumination) and labeled RBCs (laser-emitted light) at several Hcts (from 3% to 37%) for a constant average velocity of approximately 0.2 mm/s.

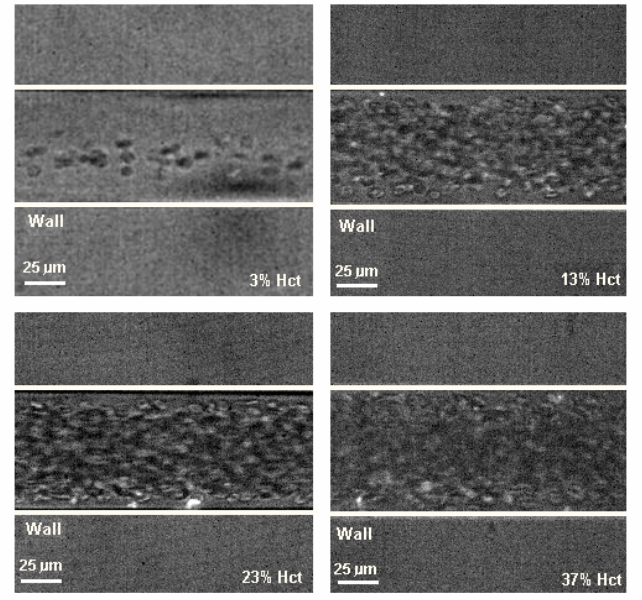

Figure 4. Normal and labeled RBCs (bright spots) with 3% Hct, 13% Hct, 23%Hct, 37% Hct (20×, 1.6 zoom).

Examination of Figure 4 reveals an overall reduction of the thickness of the cell-free layer as Hct is increased. This is further confirmed by the results obtained using the image analysis techniques described in Section 2.3. The cell-free layer thickness determined using Method 1 is shown in Figure 5. It is clear that the cell-free layer becomes narrower as Hct increases. In particular, the thickness decreases almost four fold as Hct is increased from 3% to 37%.

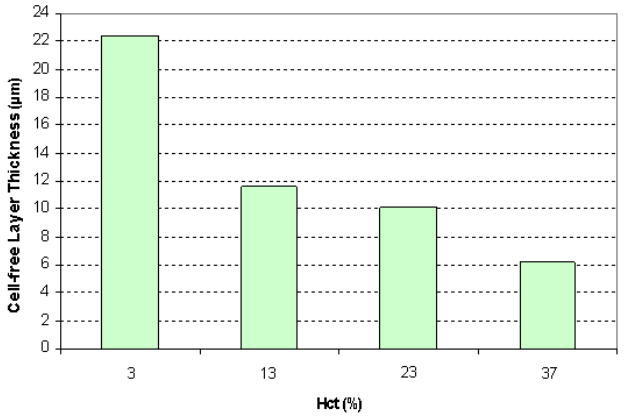

Figure 5. Average thickness of the cell-free layer at several Hcts obtained by Method 1.

In Figure 6, we compare the results in dimensionless form obtained using both methods. The overall tendency is captured in both cases and good agreement is observed between the two methods. The small discrepancies might be due to the manual analysis from the recorded images, which is involved in both methods. A slightly bigger difference is seen for the sample of 3% Hct. In this particular case, there is a small number of RBCs flowing through the microchannel and it is difficult to identify a clear boundary to the cell-free layer. Consequently, the measurements errors in this case are considerably larger. As future work a more detailed statistical comparison of both methods needs to be performed (e.g. linear regression analyses) in order to evaluate if there is any systematic difference between the two methods.

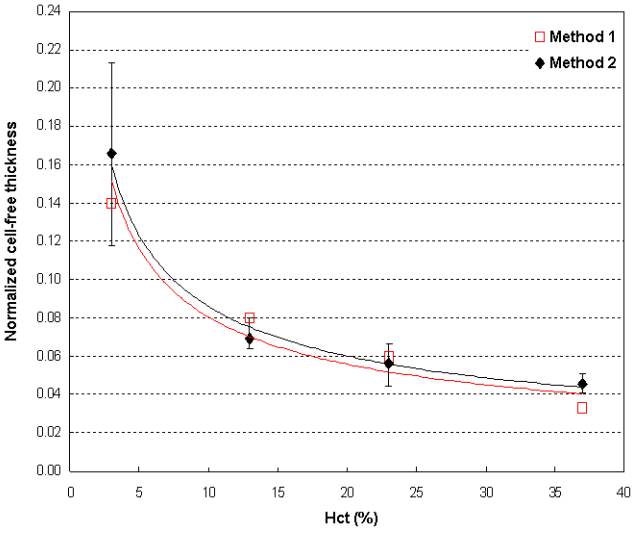

Figure 6. Comparison of the cell-free layer thickness determined using different image analysis methods. The lines are a guide to the eye.

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